# Subconjunctival Injection of Bevacizumabfor Corneal Neovascularization after Penetrating Keratoplasty, A Prospective, Open Label,Non-comparitive Study

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**Abstract :**

**Objectives :** To evaluate the efficacy of sub conjunctival Anti-VEGF Bevacizumab in post keratoplasty corneal neo vascularization in terms of graft survival.

**Study design :** Prospective ,open label Non-comparitive study.

**Participants :** A total of 17 eyes of 17 post PK patients with more than two quadrant corneal neo vascularization were administered 3 doses of 2.5 mg/0.1ml of sub conjunctival Bevacizumab each at monthly intervals, starting from first dose been given on day of enrollment and followed up for a minimum period of 6 months.

**Outcomes : Primary outcomes:**

- Reduction in the number of segments involved
- Change in the number of preexisting corneal vessels crossing Graft-Host Junction.

**Secondary outcomes :** Effect on Graft clarity, CCT, visual acuity and side effects related to drug.

**Results :** From baseline visit to the last follow up visit, the mean reduction in the number of vascularised corneal segments was 47% in patients having 4 quadrant (13-16 segments) CoNV and 75% in patients having 3 quadrants(9-12 segments) CoNV. In 35% patients all corneal vessels crossing graft host junction receded. Out of 17 cases,11 patients had Graft clarity of +4 ,at the end of 6th month follow up.(p=0.0017significant) and 13 patients (76.47%) were with a central corneal thickness less than 600 micrometer (. p=0.0005 significant). Visual acuity showed no significant change. No local/ systemic adverse reaction was reported during the study period.

**Conclusion :** Sub conjunctival Bevacizumab was found to be effective as adjuvant in Post Penetrating Keratoplasty Corneal neo vascularization. Drug is well tolerated in most of the patients without any local or systemic side effects.

**Key Words :** CoNV (Corneal neo vascularization), Bevacizumab, CCT(central corneal thickness), GHJ (graft host junction)

## Introduction :

Keratoplasty (corneal transplantation) is a surgical procedure in which the diseased cornea is replaced with a healthy donor cornea. Being an immunologically privileged structure, Corneal Transplantation is considered as the most common and successful form of human solid tissue transplantation.[1,2] Corneal neo vascularization is an adverse factor for the success of Penetrating Keratroplasty, with the survival rates been less than 50% even with local and systemic immunosuppression.[3]

Depending on the number of quadrants involved, Corneal Neo vascularization may be classified into low risk(1 quadrant),medium risk (2 quadrant), or high risk (more than 2 quadrant).[4]

Current treatment modalities for treating CoNV include medications, such as steroids or non-steroidal anti-inflammatory agents, laser photo coagulation, fine-needle diathermy, photo dynamic therapy or restoration of the ocular surface with the use of conjunctival, limbal, or amniotic membrane transplantation. These have demonstrated variable and largely limited clinical success.[5,6] Furthermore, none of these treatments specifically target the molecular mediators of angiogenesis.

## Role of anti-vegf in corneal vascularization :

VEGF is a member of a family of proteins, which include VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. Among these, VEGF-A isoforms have received the most attention as mediators of pathologic CoNV.[9] VEGF-A is known to increase migration and mitosis of endothelial cells, increase methane mono-oxygenase activity, and play a role in the creation of new blood vessel and vessel fenestrations. Vascular endothelial growth factor inhibitors are emerging as the new pharmacological therapy in the management of post

keratoplasty graft rejection. Bevacizumab is FDA approved for intravenous administration in the treatment of various cancers, is a full-length, humanized murine monoclonal antibody with a molecular weight of 149kD. Bevacizumab recognizes all isoforms of VEGF and recently it's off label use has also been considered as a new treatment modality for CoNV.[7,8]

Toevaluate the efficacy and safety of subconjunctival Bevacizumab on CoNV in Post Kerato plastypatients, a prospective, non randomized open label study was done in the Department of Ophthalmology, S.N. Medical College from Feb 2018 to July 2019.

## Material and Methods :

A total of 17 eyes of 17 patients were enrolled for study.

**Inclusion criteria :**

- Patients with corneal neo vascularization post PK(more than 2 quadrants).
- The patient who signed the consent to be in the regular follow up / treatment.

**Exclusion criteria :**

- Patientshaving uncontrolled Glaucoma.
- Active Inflammation/infection in eye
- Poor Corneal Epithelialization
- Patient, not suitable for Bevacizumab use viz uncontrolled systemic hypertension, recent Myocardial Infarction , recent CVA , stroke and Pregnant females .

## To assess vascularization :

Corneal photographs were taken with slit lamp mounted digital camera. For every patient a computerized grid was made, in which the whole of the corneal surface was divided into 16 equal segments and number of segments having vascularization were noted along with the number of vessels crossing Graft Host junction. (Figure1)

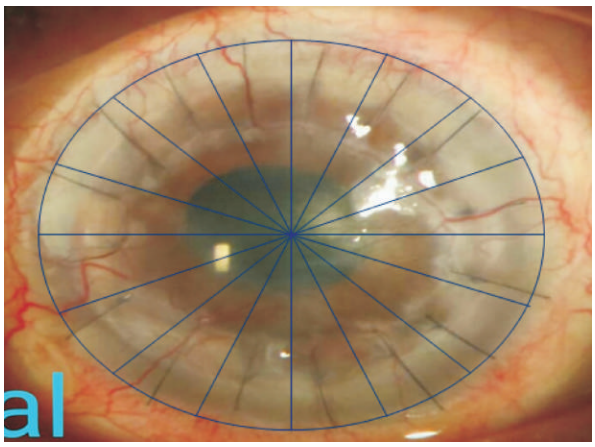

*Figure 1 : Computarised grid made on Coraea*

*Table 1 : Base line Characterstics*

| Base Line Characterstics | No. of Pts. (17) |
|---|---|
| **1. Age :** | |
| 0-20 | 01 |
| 21-40 | 08 |
| 41-60 | 03 |
| 60-80 | 05 |
| **2. Gender :** | |
| Male | 11 |
| Female | 06 |
| **3. Background diseaseFor which PK / Triple was done :** | |
| Corneal scarring(Trauma/ulcer) | 10 |
| PBK (Decompensated Cornea) | 04 |
| Infective keratitis | 02 |
| Degeneration | 01 |
| **4.CoNV (number of segments ) at time of enrollment (post PK)** | |
| 9-12 segments | 04 |
| 13-16 segments | 13 |

After taking Informed consent, under aseptic condition, 3subconjunctivalinjections of 2.5 mg/.1 ml Bevacizumab were given to all 17patients at monthly intervals starting from day of enrollement and followed up for a period of minimum 6 months.

The Primary outcome in our study was the assessment ofthe effect of sub conjunctivalinj. (0.25 ml) of Bevacizumab on CoNV, by the following variables .

- Reduction in number of segments involved.
- Change in the number of preexisting Corneal vessels crossing Graft Host Junction.
- Appearance of any new blood vessel

The secondary outcomes were the effect of sub conjunctival Bevacizumabon Graft Clarity, CCT and Visual Acuity and side effects related to sub conjunctival Bevacizumab.

## Statistical analysis :

Data was analyzed using Microsoft excel 2010. Paired student's t-test were used for hypothesis testing of grouped values of pre injection and 1, 3 and 6 month follow-up viz vessels segments , vessels crossing Graft Host junction, visual acuity, CCT and Graft clarity. A p-value <0.05 was considered statistically significant.

**Results :**

17 eyes of 17 patients (12 males and 7 females) were included in this study. The demographic characterstic of the study population including age, gender, eye , background disease for which keratoplasty was done and severity of CoNV(post PK) at the time of enrollment are listed in table 1

All 17 patients were followed up for a minimum period of 6 month.

From baseline visit to the last follow up visit, out of 17 patients the mean reduction in the number of vascularised corneal segments was 47% in 13 patients with 4 quadrant (13-16 segments) CoNV and 75% in rest 4 patients, with 3 quadrants(9-12 segments) CoNV, (table 2). So overall out of 17 pts 9 patients showed significant reduction in the number of segments involved (p=0.002 at 6 month significant).(Figure2)

Before injection all 17 patients were having at least some blood vessels crossing graft host junction, though in most of them, number was <10. At the end of 6 month follow up in 6 patients Graft Host Junction became free of CoNV. (p=0.002, significant)(table3) (Figure 3)

In our study we have noticed the improvement in Graft Clarity after sub conjunctival injection of Bevacizumab in significant number of patients. Before injection we had only 4 patients with grade 4 + Graft Clarity, which increased to 11 patients by the end of 6 month study period.(p=0.0017; significant).

Significant number of patients also showed improvement in CCT from baseline visit to final visit. Before injection only 5 patients had CCT <600 microns, which increased to 13 patients at the end of 6 month F/U. (p=0.0005 significant).

Visual acuity did not show any significant improvement /change from base line to final follow up visit.

No systemic or ocular side effect is noted during the entire study period.

*Table 2 : Segment wise involvement of CoNV.*

| NUMBER OF VASCULARIZED SEGMENTS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Timings** | | **13-6** | **%** | **9-12** | **%** | **5-8** | **%** | **0-4** | **%** |
| **BEFORE INJ** | | 13 | 76.47 | 4 | 23.52 | 0 | - | 0 | - |
| **F/U** | **1 MONTH** | 6 | 35.29 | 5 | 29.41 | 4 | 23-52 | 2 | 11.76 |
| | **3 MONTH** | 6 | 35.39 | 2 | 11.76 | 5 | 29.41 | 4 | 23.52 |
| | **6 MONTH** | 7 | 41.17 | 1 | 5.88 | 6 | 35.29 | 3 | 17.64 |

*Table 3 : CoNV with respect to Graft Host Junction.*

| NUMBER OF VESSELS CROSSING GRAFT_HOST JUNCTION | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Timings** | | **>20** | **%** | **16-20** | **%** | **11-15** | **%** | **6-10** | **%** | **1-5** | **%** | **0** | **%** |
| **BEFORE INJ** | | 1 | 5.88 | 2 | 11.76 | 1 | 5.88 | 6 | 35.29 | 7 | 47.18 | 0 | - |
| **F/U** | **1 MONTH** | 1 | 5.88 | 0 | - | 3 | 17.65 | 5 | 29.41 | 5 | 29.41 | 4 | 23-52 |
| | **3 MONTH** | 1 | 5.88 | 0 | - | 1 | 5.88 | 4 | 23.52 | 5 | 29.41 | 6 | 35-39 |
| | **6 MONTH** | 1 | 5.88 | 0 | - | 0 | - | 5 | 29.41 | 5 | 29.41 | 6 | 35-39 |

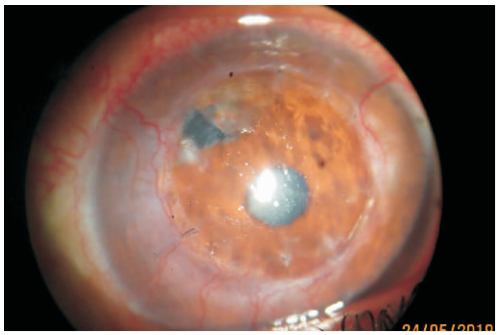

*Figure 2a :Baseline pic (8month Post PK with 360 degree CoNV)*

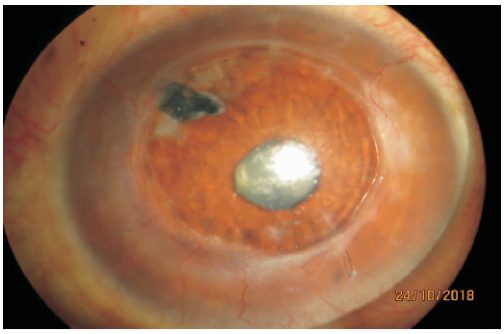


*Figure 2b : 2 months after 3rd dose of sub conjunctival 0.25 mlBevacizumab (5 month F/U visit). significant decrease in the caliber and length of preexisting vessels is noted and no new blood vessel is seen .BCVA 6/24p*

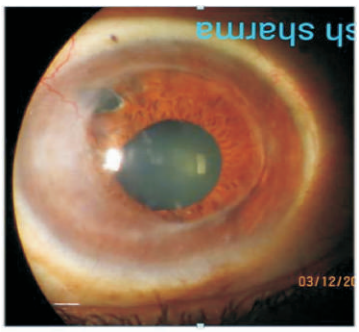

*Figure 2 c : Graft clarity 4+and BCVA 6/24p maintained after 7 month of Follow up.*

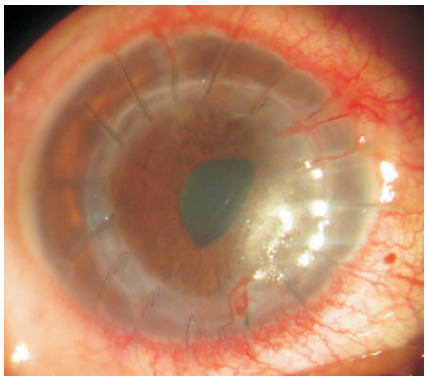

*Figure 3 a : (Baseline pic before subconjunctival injection of Bevacizumab) Patient presented with signs of rejection . UCVA 6/60*

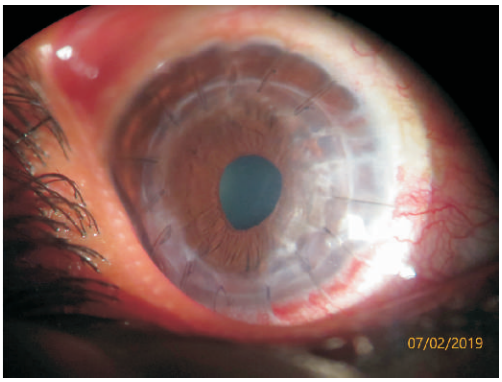


*Figure 3b : 4 month post PK with 270 degree CoNV*

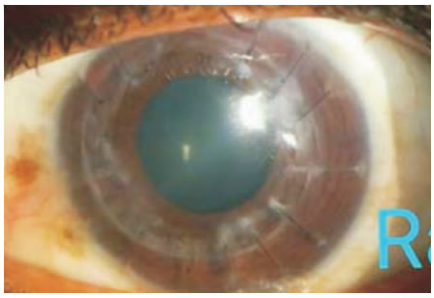

*Figure 3c : After 6 month follow up*

*Table 4 : Graft clarity before injection and at follow up visits*

| GRAFT CLARITY | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | % | 1+ | % | 2+ | % | 3+ | % | 4+ | % |
| BEFORE INJ | | 0 | 0 | 1 | 5.88 | 2 | 11.76 | 10 | 58.82 | 4 | 23.53 |
| F/U | 1 MONTH | 0 | 0 | 1 | 5.88 | 1 | 5.88 | 4 | 23.53 | 11 | 64.71 |
| | 3 MONTH | 0 | 0 | 1 | 5.88 | 1 | 5.88 | 4 | 23.53 | 11 | 64.71 |
| | 6 MONTH | 0 | 0 | 1 | 5.88 | 1 | 5.88 | 4 | 23.53 | 11 | 64.71 |

*Table 5 : CCT before injection and on follow up*

| CENTRAL CORNEAL THICKNESS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 401-500 | % | 501-600 | % | 601-700 | % | 701-800 | % | >800 | % |
| BEFORE INJ | | 0 | - | 4 | 23.52 | 6 | 35.29 | 5 | 35.29 | 2 | 11.76 |
| F/U | 1 MONTH | 3 | 17.64 | 6 | 35.29 | 6 | 35.29 | 0 | - | 1 | 5.88 |
| | 3 MONTH | 5 | 29.41 | 9 | 52.94 | 1 | 5.88 | 1 | 5.88 | 1 | 5.88 |
| | 6 MONTH | 3 | 29.41 | 8 | 47.05 | 1 | 5.88 | 1 | 5.88 | 1 | 5.88 |

*Table 6 : Visual acuity before injection and on follow up*

| VISUAL ACUITY (logMAR) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5-0.8 | % | 0.9-1.2 | % | 1.3-1.5 | % | 1.6-1.8 | % | 1.9-2.1 | % |
| BEFORE INJ | | 3 | 17.64 | 9 | 52.94 | 4 | 23.52 | 0 | - | 1 | 5.88 |
| F/U | 1 MONTH | 2 | 17.64 | 8 | 47.05 | 5 | 29.41 | 0 | - | 1 | 5.88 |
| | 3 MONTH | 5 | 29.41 | 7 | 41.11 | 1 | 5.88 | 3 | 17.64 | 1 | 5.88 |
| | 6 MONTH | 7 | 41.11 | 4 | 23.52 | 2 | 11.76 | 1 | 5.88 | 3 | 17.64 |

*p=0.012 (non significant)*

## DISCUSSION :

Corneal neovascularization is a risk factor for graft failure and rejection after keratoplasty supported by Bachmann B ,et al.[9]

In our study we observed the efficacy of sub conjunctival AntiVEGF Bevacizumabin patient with post operative Corneal neo vascularization (more than 2 quadrants) in terms of Graft survival.

Most of the cases included in our study were young with 8 (47.05%) cases in the age group of 21-40 years. There was a male preponderance in our cases. The leading indications for Penetrating Keratoplasty in our study were corneal scarring post trauma or ulcer (59%), supported by LaxmanDasar et al[10]in 2013. They had studied the indications of penetrating keratoplasty in Southern India.

To quantify CoNV we prepared a computerized Grid, dividing cornea into 16 equal segments. The parameters we noted down were number of segments involved with CoNV, number of blood vessels crossing Graft Host junction, CCT, Graft Clarity and Visual Acquity.

At the time of enrollment, out of 17 patients,13 pts were having CoNV in all 4 quadrants (13-16). Rest 4 patients were having 3 quadrant CoNV between 9-12 segments .There is a statistically significant decrement in vessels post injection when compared to pre injection in both the groups .In our study ,the maximum effect was noted at1 month after sub-conjunctival injection of Bevacizumab supported by TSchollmayer et al (2008) who performed a retrospective case series study of nine eyes of nine patients with corneal transplant and neo vascularization. They followed up all the patients till 6 months and concluded that topical and sub conjunctivalbevacizumab is effective in regressing neo vascularization in keratoplastypatients .[11]

Lochab D, et al. in 2018[12]also evaluated the effect of sub conjunctival anti-VEGF on corneal neo vascularization post penetrating keratoplasty. In all patients who were subjected to subconjunctivalBevcizumab, the regression of neovascularization at 1 week, 4 weeks and 6 weeks were noted and there was a significant decrease in neo vascularization in central segments 1.97± 3.72 as compared to pre 5.[13] ±7.12(p=0.001) and also in peripheral segments at 6 weeks 29.90 ± 15.73 as compared to pre Bevacizumab .

The second parameter we evaluated was the number of vessels, crossing graft host junction before injection and on follow up visits. All cases at base line had at least some vessels crossing Graft Host (in most of cases number was less than 10). At last follow up ,in 6 patients (35.29%) GHJ was free of CoNV. There is also a decrease in length and caliber of vessels. Our results are similar to those of Bahar et al. who reported partial regression of corneal vessels using 2.5 mg/0.1 Bevacizumab in seven patients with CoNV(13).

In our study we found significant improvement of Graft clarity and which was maintained till the last follow up. Before injection, we had only 4 patients with grade 4 + Graft Clarity, which by the end of study period had increased to 11 patients with grade 4+ clarity. We can conclude that regression of corneal neo vascularization enhances the Graft Clarity.

We also noted significant reduction in CCT after sub conjunctival Bevacizumab injection. Only 4 patients were having normal central corneal thickness (<600 microns) prior to injection, which increased to 13 at the end of 6 month follow up.[14] No ocular or systemic complications were noted during study period. You et al[15] reported that pain at the subconjunctival injection site in nine eyes (31.0%), subconjunctival hemorrhages in eight eyes (27.6%), and ocular irritation in one eye (3.4%). There were no systemic complications, such as increased blood pressure or transient ischaemia, in their study. Krizova et al [16] also reported tiny epithelial corneal defects in three patient's post subconjunctivalBevacizumab. Erdurmus et al have reported

dry eye in one patient after subconjunctivalBevacizumab injection (2.5 mg/0.1 mL).[17]

## Conclusion :

The use of sub conjunctival Bevacizumab seems to be an effective adjuvant therapy for Corneal Neo vascularisation, especially in cases which are unresponsive to conventional antiin flammatory therapy. sometimes repeated injection are required to augment or in cases of recurrence of CoNV but it is proved to be safe .Our study has small follow up period so long follow up studies are needed to confirm its definite role as key therapeutic agent in the inhibition of corneal neo vascularisation.

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## Charles William Simcoe

The Simcoe Cannula was developed about 40 years ago by C. William Simcoe MD, an ophthalmologist in Oklahoma, USA. Bill Simcoe was born in Still water on June 5, 1931 and passed away in his Tulsa home on October 22, 2017.

Dr. Simcoe also developed many innovations such as Simcoe irrigation and aspiration cannula & C-loop haptics. While examining and reshaping a paper clip, he had an idea of how to invent a much safer intraocular lens design the Simcoe open C loop which has become the industry standard in modern cataract surgery. He refused to patent any of his inventions and are widely used now in cataract surgery

A native of Still water, Charles William Simcoe was a Korean War and Marine Corps veteran. He was a graduate of the University of Oklahoma medical school. Through Project Orbis, a nonprofit dedicated to preventing blindness, Simcoe travelled the globe, teaching doctors how to perform safer, less costly cataract surgeries.